Anti-anxiety activity of hydro alcoholic extract of *Scoparia dulcis* Linn. assessed using different experimental anxiety models in rodents

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Abstract

*Scoparia dulcis* belonging to the family Scrophulariaceae is a valuable medicinal herb, had showed antiviral, antimarial, anticancer and antidiabetic activities. The present study was aimed to investigate the anti-anxiety activity of crude hydroalcoholic extract of *S.dulcis* L by various behavioural models. Preliminary phytochemical investigation revealed the presence of phenols and flavonoids. The extract at 100mg/kg and 200mg/kg was evaluated for anti anxiety activity by Open-field test [OFT], Elevated plus-maze test [EPM], Elevated Zero-maze test [EZM], Social interaction test [SI] And Novelty induced suppressed feeling latency test [FL] and the results of behavioral tests indicated the dose dependent anti-anxiety activity of *Scoparia dulcis* which is comparable to standard. It was concluded that crude hydroalcoholic extract showed anti anxiety activity. Further studies are needed to identify the anxiolytic mechanism(s) and the phytochemicals responsible for the observed anxiolytic effect of the hydroalcoholic extract of *Scoparia dulcis*.

Keywords: *Scoparia dulcis*, Elevated plus-maze test, Open-field test, Scrophulariaceae

1. Introduction

Now a day’s neuropharmacological research occupied leading position in pharmaceutical drug development industry[1]. Anxiety is a most prevalent disorder in younger individual’s, characterized as concern or fear about some defined or undefined future threat and emotional behavior, unpleasant mood, uneasiness and discomfort associated with disability in both educational and professional life[2][3]. Anxiety exhibited negative impact on quality of life and increases suicidal behavior in individuals[4][5]. Treatment along with improvement of quality of life is an important task and use of clinically available anti anxiety agents in the market offered limited therapeutic benefit because of their adverse drug effects, dietary restrictions and drug interactions[6]. These are some of the reasons that lead researchers towards potent and safest anti anxiety agent’s development.

Experimental works revealed role of herbal medicines in the treatment of psychological problems includes anxiety, depression and other psychological problems[7][8]. Evaluation of Antianxiety activity of herbal medicine research was increased now a day’s like antianxiety activity of *Camellia sinensis, Nimphea alba* Linn, *Foeniculum vulgare, Magnolia Bark, Vitex Negundo* Linn,etc.[9]-[12].

Pharmacologically weeds had shown antibacterial activity[13] central nervous disorders[14], antidiabetic activity, Hepatoprotective Activity, Antimicrobial Activity, Wound Healing Activity, Anti-Cancerous Activity, Immunomodulatory Activity, Hypotensive effect[15]. Therefore, the present research was designed for evaluation of anxiolytic effect of *Scoparia dulcis* belonging to the family Scrophulariaceae is an valuable medicinal herb, commonly called as broom weed. Its vernacular names are sarkaraivembu or neernangai [Tamil], Peshanabheda [Sanskrit] and Kallurukki [Malayalam]. *S.Dulcis* had offered excellent pharmacological uses include antiviral activity, antimalarial activity[16], anticancer activity[17] and antidiabetic activity[18]. The plant contains various kinds of biochemical compound such as, phenols, saponins, tannins, amino acids, flavonoids, terpenoids and catecholamines[19].

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It is an invasive weed widely distributed in many tropical countries and abundantly in Cambodia, Laos, Java, Sumatra, Vietnam, Taiwan, South America and Amazon rain forests[20]. Our present study is designed with an aim to evaluate the anxiolytic activity of crude ethanolic extract of *S. dulcis* L on rats.

2. Materials and methods

2.1 Animals

Adult Swiss wistar albino rats of either sex [200-250gms] were procured from Central Animal House, Banaras Hindu University, Varanasi [Regd.No.-542/02/ab/CPCSEA] and were randomly distributed to different groups before experimental work. They were kept in polypropylene bags and were maintained for 12hrs light / dark cycle at an ambient temperature [25°C ±1°C] and fed with laboratory chow pellets [Hindustan lever] and water *ad libitum*. The experimental protocol was duly approved by Institutional ethical committee.

2.2 Plant material

The whole plant, *S. dulcis* L was collected from Acharya N.G.Ranga Agricultural University, Muthukur Road, Nellore and was authenticated by Dr. S. Md. Khasim, Head, Department of Botany, Acharya Nagarjuna University, and Guntur. A plant specimen was also planted in the medicinal garden of Narayana Pharmacy college and a voucher specimen [BN/PE/008] was also submitted to the head of the Institution.

2.3 Preparation of plant extract

The whole plant of *S. dulcis* were collected and washed with water. They were dried in shade and fumigated. They were powdered by pulveriser and extracted with ethanol [90%] for 48 hours by using soxhlete apparatus. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure using rotary evaporator was used for anxiolytic activity. A preliminary phytochemical investigation was carried out before conducting the bioassay.

2.4 Experimental Work

2.4.1 Anxiolytic activity

*Drug treatment*

Before the experimental work, an acute toxicity of hydro alcoholic extract of *S. dulcis* L was evaluated in rat as per OECD Guideline 423 before conducting the experiment. Three rats in each group (weight: 200-250 g, age: 8-12 weeks) with four groups were divided based on drug dose dependent and the minimum and maximum doses were reported as 100 mg/kg and 200 mg/kg. Both the doses were administered orally by gavage. The hydroalcoholic extract [50%] of *S. dulcis* L was dissolved in 0.2% of carboxy methyl cellulose [CMC] suspension prior to oral administration. The extract was administered by using or gastric cannula in the doses of 100 and 200 mg/kg once daily for three consecutive days. Lorazepam [0.5 mg/kg, UCB Pharma limited, India] was used as the standard drug to test anxiolytic activity and was administered to one group rats 30 minutes before experimentation for comparison. Control rats were treated with the vehicle [0.2% CMC suspension in distilled water]. Experiments were conducted on third day after drug administration of one hour.

*Behavioral testing’s*

**Open-field test [OFT]**

In this test the open field was prepared by using plywood and consisted of squares [61×61cm]. The apparatus was painted black except 6mm thick white lines which divided the floor into 16 squares. Open field was lighted by a 40 watts bulb focusing into the field from a height of about 100cm. the entire room except the open field was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 minutes and the following behavioral aspects were noted i.e Ambulation, Rearings, Self grooming, Activity in centre, and Fecal droppings[21].

**Elevated plus-maze test [EPM]**

The maze had two arms, 50 × 10cm, crossed with two closed arms having same dimension but having 40cm high walls. The arms were connected with a central square, 10 ×10cm giving the apparatus shape of a plus sign. The maze was kept in a dimly-lit room and elevated 50cm above the floor. The rats were placed in the individually in centre of the maze, facing an enclosed arm. Thereafter number of entries and time spent on the open and closed arms were recorded during the next 5 minutes. An arm entry was defined when all four paws of the rat were in the arm. Observations were made by neutral blind observer[22].

**Elevated Zero-maze test [EZM]**

The maze had black Perspex annular platforms [105cm in diameter, 10cm width] elevated to 65cm above the ground level, divided equally into four quadrants. The two opposite quadrants were enclosed by a black Perspex wall [27cm high] on both the inner and outer edges of the platform, while the remaining two opposite quadrants were
surrounded by perspex lip [1cm high] which served as a tactile guide in animals on these open areas. The apparatus were illuminated by dim light arranged in such a manner as to provide similar lux levels in open and enclosed quadrants. The rats were placed on one of the enclosed quadrants for a 5 minutes test period. The maze was cleaned between test sessions. During the 5 minutes test period, time spent on open arms, number of head dips over the edges of the platform and number of the stretched attend postures from closed to open quadrants were recorded. Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area only when all the four paws had passed the open closed divide.[23]

Social interaction test [SI]

The rats were first housed individually for 5 days before performing the test. The apparatus used for the test was a wooden box [60x60x35cm] with a solid floor and was placed in a dimly lit room. On day 6, the rats were placed individually in the box and given two 7.5 minutes familiarization sessions at 2 hr interval. On day 7, rats were paired on weight and sex basis and placed in the box for 7.5 minutes. During this time, total time spent by the rat pair in social interactions including sniffing, following, grooming, kicking, boxing, biting and crawling under or over the partner was recorded by a neutral blind observer.[24]

Novelty induced suppressed feeling latency test [FL]

The test apparatus was a wooden box [60x60x35cm] with a solid floor placed in a dimly lit room. The floor of the wooden box was covered with 2cm layer of wooden chips and laboratory chow pellet was placed on the floor. A similar arrangement was made in the home cages of the rats. Food was removed from the home cage 48hr prior to testing but they were under adlibitum. The rats were placed individually in the test chamber and the latency to begin eating was recorded. If the rat had not eaten within 300secs the test was terminated and a latency score 300secs was assigned. Observations were made by neutral blind observer.[25]

3. Results and Discussion

3.1 Anxiolytic activity

Open field exploratory behavior

Both the doses of S.dulcis L extract on rats showed a significant increase in open field ambulation, rearings, self grooming and activity in centre in comparison to vehicle treated rats evidencing significant anxiolytic activity with the standard. But the fecal dropings found to be unchanged [Table 1].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Ambulation</th>
<th>Rearings</th>
<th>Self grooming</th>
<th>Activity in centre</th>
<th>Fecal droppings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>47.28±3.40</td>
<td>8.64±1.98</td>
<td>6.58±1.76</td>
<td>1.36±2.11</td>
<td>3.85±1.73</td>
</tr>
<tr>
<td>S.dulcis100 (mg/kg)</td>
<td>6</td>
<td>72.01±12.68</td>
<td>10.00±1.68</td>
<td>7.86±1.86</td>
<td>2.56±1.72</td>
<td>2.65±0.75</td>
</tr>
<tr>
<td>S.dulcis200 (mg/kg)</td>
<td>6</td>
<td>82.88±5.12</td>
<td>12.16±2.08</td>
<td>6.94±1.50</td>
<td>3.62±2.88</td>
<td>3.06±1.01</td>
</tr>
<tr>
<td>Lorazepam (0.5mg/kg)</td>
<td>6</td>
<td>84.66±2.55</td>
<td>17.16±1.50</td>
<td>10.86±2.16</td>
<td>6.12±1.34</td>
<td>2.12±0.75</td>
</tr>
</tbody>
</table>

Values are means SD. "a" indicates statistical significance respectively in comparison to vehicle, ethanolic extract and lorazepam treatments. "aa" denotes P<0.01 in comparison to vehicle (ANOVA followed by Students t-test), "ab" denotes P<0.05 in comparison to vehicle (ANOVA followed by Students t-test), "ab" denotes P<0.01 in comparison to vehicle (ANOVA followed by Students t-test).

Elevated plus maze behavior

The extract treated rats exhibited dose dependent significant increase in time spent in open arms, entries made in open arms and significant decrease in time spent in enclosed arms and entries in enclosed arms comparing to control rats. The results obtained by open/closed time and entries ratios also indicated significant anxiolysis in rats by the S.dulcis extract. LR caused more anxiolysis when compared to the extract [Table 2].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Time spent on (secs)</th>
<th>Entries on</th>
<th>Ratio of open/enclosed arms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Enclosed arms (secs)</td>
<td>open arms</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>213.87±4.36</td>
<td>29.76±1.98</td>
<td>7.65±2.86</td>
</tr>
<tr>
<td>S.dulcis100 (mg/kg)</td>
<td>6</td>
<td>153.78±24.56</td>
<td>63.89±7.76</td>
<td>10.89±1.46</td>
</tr>
<tr>
<td>S.dulcis200 (mg/kg)</td>
<td>6</td>
<td>144.36±13.28</td>
<td>68.72±1.67</td>
<td>12.79±2.35</td>
</tr>
<tr>
<td>Lorazepam (0.5mg/kg)</td>
<td>6</td>
<td>163.25±3.65</td>
<td>69.76±2.48</td>
<td>10.86±2.16</td>
</tr>
</tbody>
</table>

Values are means SD. "a" indicates statistical significance respectively in comparison to vehicle, ethanolic extract (100mg/kg) and lorazepam treatments. "aa" denotes P<0.01 in comparison to vehicle (ANOVA followed by Students t-test), "ab" denotes P<0.05 in comparison to vehicle (ANOVA followed by Students t-test).
Elevated zero maze behavior

The rats treated with the extract showed anxiolysis in terms of significant increase in time spent in open arms, entries in open arms and number of head dips on this paradigm. However the response stretched postures remain unchanged. LR caused significant anxiolysis and effects were comparable to the extract [Table 3].

Table 3: Effect of ethanolic extract of S. dulcis on the elevated zero maze behaviour in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Time spent on open arms</th>
<th>Head dips</th>
<th>Stretched attend postures</th>
<th>Entries in open arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (100 mg/kg)</td>
<td>6</td>
<td>68.16±3.63e</td>
<td>11.87±2.97a</td>
<td>3.15±0.97</td>
<td>8.23±1.27abc</td>
</tr>
<tr>
<td>S. dulcis200 (mg/kg)</td>
<td>6</td>
<td>71.89±2.57a</td>
<td>12.98±3.46b</td>
<td>2.77±1.56c</td>
<td>2.89±1.37abc</td>
</tr>
<tr>
<td>Lorazepam (0.5mg/kg)</td>
<td>6</td>
<td>75.37±1.76a</td>
<td>13.27±1.53bc</td>
<td>3.89±0.24</td>
<td>11.56±1.35abc</td>
</tr>
</tbody>
</table>

Values are mean± SD

a, b, c indicate statistical significance respectively in comparison to vehicle, ethanolic extract (100mg/kg) and lorazepam treatments.

aa, bb, a, b, c denotes P<0.05 in comparison to vehicle (ANOVA followed by Students t-test)

Social interactions

The rats treated with the extract spent significantly more time in social interactions in comparison to control rats and the effects were found to be dose dependent. LR also caused significant increase in this paradigm and effects were comparable to that of higher dose [200 mg/kg] [Table 4].

Table 4: Effect of hydroalcoholic extract S. dulcis in the social interaction test in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n(pair)</th>
<th>Social interaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (home cage)</td>
<td>12</td>
<td>146.98 ±1.87</td>
</tr>
<tr>
<td>S. dulcis100 (mg/kg)</td>
<td>6</td>
<td>209.31±8.54abc</td>
</tr>
<tr>
<td>S. dulcis200 (mg/kg)</td>
<td>6</td>
<td>245.09±2.56abc</td>
</tr>
<tr>
<td>Lorazepam (0.5mg/kg)</td>
<td>6</td>
<td>238.67±5.76abc</td>
</tr>
</tbody>
</table>

Values are mean± SD, a, b, c indicate statistical significance respectively in comparison to vehicle, ethanolic extract (100mg/kg) and lorazepam treatments.

aa, bb, cc, dd denote P<0.05 and P<0.01 in comparison to vehicle (ANOVA followed by Students t-test) respectively.

Novely induced suppressed feeding latency

The extract caused dose dependent significant attenuation of novely induced suppressed feeding latency in rats when compared to vehicle treatment. LR also caused similar effects [Table 5].

Table 5: Effect of hydroalcoholic extract of S. dulcis in the novelty induced suppressed feeding latency test in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n(pair)</th>
<th>Social interaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (home cage)</td>
<td>12</td>
<td>87.98 ±1.06</td>
</tr>
<tr>
<td>Vehicle (novel cage)</td>
<td>12</td>
<td>148.98 ±0.87e</td>
</tr>
<tr>
<td>S. dulcis100 (mg/kg)</td>
<td>6</td>
<td>124.31±8.54abc</td>
</tr>
<tr>
<td>S. dulcis200 (mg/kg)</td>
<td>6</td>
<td>112.78±2.56abc</td>
</tr>
<tr>
<td>Lorazepam (0.5mg/kg)</td>
<td>6</td>
<td>98.56±2.31d</td>
</tr>
</tbody>
</table>

Values are mean± SD, a, b, c, d indicate statistical significance respectively in comparison to vehicle (home cage), vehicle(novel cage), ethanolic extract (100mg/kg) and lorazepam treatments.

aa, bb, cc, dd denotes P<0.05 in comparison to vehicle (ANOVA followed by Students t-test)

Experimental results supported hydroalcoholic extract exhibited anxiolytic activity in dose dependent manner [S. dulcis100 mg/kg, S. dulcis200 mg/kg] which is comparable to that of standard in all the experimental methods. Scientifically, it was proved that plant metabolites like Phenols and flavonoids plays vital role in the disease management and treatment of anxiety[26]. Jung et al[27]in 2006 reported anxiolytic activity of Gastrodia elata mainly due to the presence of phenols, Jong et al[28] in 2007 revealed the role of phenols in the management of anxiety disorders and flavonoids role in the treatment of anxiety were reported by Eliana et al[29] in the year 2008. Flavonoids, essential oils, phenolic acids, and alkaloids containing plants indicated as anxiolytic agents[29]. Therefore, the dose dependent antianxiety activity of S. dulcis was supported by the phytochemical screening reports, revealed the presence of phenols and flavonoids in hydroalcoholic extract.

4. Conclusion

In our present, we revealed the antianxiety activity of hydroalcoholic extract of S. dulcis at 100 & 200 mg/kg dose level. The results were comparable to that of standard and control group. Further work is needed for the evaluation of isolated compounds activities.
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References


